Biochem. J. (1966) **99**, 604

C-Glycosylflavonoids

THE CHEMISTRY OF ASPALATHIN

BY B. H. KOEPPEN
Department of Food Science, University of Stellenbosch, South Africa
AND D. G. ROUX
Leather Industries Research Institute, Rhodes University, Grahamstown, South Africa

(*Received* 23 *November* 1965)

1. The isolation of aspalathin, the principal phenolic constituent in the leaves of *Aspalathus linearis*, is described and its properties are discussed. 2. The compound has been identified as 3′-*C*-β-D-glucopyranosyl-2′,3,4,4′,6′-pentahydroxydihydrochalcone by the preparation and analysis of various derivatives, by photochemical oxidation to 2,3-dihydroiso-orientin and by nuclear-magnetic-resonance studies.

Aspalathin is the principal monomeric polyphenol occurring in the leaves of *Aspalathus linearis* which is used in South Africa for the manufacture of rooibos tea. Initially the compound was considered to be a *C*-glycosylflavanone (Koeppen, Smit & Roux, 1962) but in a recent preliminary communication (Koeppen & Roux, 1965*a*) evidence in favour of a *C*-glycosyldihydrochalcone structure was presented.

The present paper describes the isolation and characterization of aspalathin, the preparation of various derivatives and the experimental procedures which have led to the identification of this compound as 3′-*C*-β-D-glucopyranosyl-2′,3,4,4′,6′-pentahydroxydihydrochalcone (I).

EXPERIMENTAL AND RESULTS

Infrared-absorption spectra were recorded on a Beckman IR9 spectrophotometer by the KBr-disk method (0·4–0·7 mg. of compound/400 mg. of KBr). C, H, Br, acetyl and methoxyl determinations were by K. Jones, Microanalytical Laboratory, C.S.I.R., Pretoria, South Africa. A Gallenkamp model MW-120 semi-microebulliometer was employed for the determination of molecular weights, 0·25 g. samples of the compound in acetone (10 ml.) being used according to the makers' instructions and with benzoic acid and orientin octa-acetate as standards. All melting points are uncorrected and were determined by the Kofler method. Mixed melting points were determined on molecular mixtures by the method of Roux & Maihs (1960).

Isolation and characterization of aspalathin

The procedure for the isolation of the crude polyphenols and their separation by preparative paper chromatography has been described (Koeppen *et al.* 1962). Aspalathin moved with the most mobile fraction (band III) in butan-1-ol–acetic acid–water (20:5:11, by vol.), and subsequent chromatography of this fraction in water resulted in its separation into two major components of which the more mobile (designated J) consisted of aspalathin (Koeppen *et al.* 1962). The material was eluted from the chromatograms with aq. 70% (v/v) ethanol and the eluate was evaporated to a small volume (about 40 ml.) under reduced pressure and extracted with several portions of ethyl acetate. The extracts were combined, dried over anhydrous Na_2SO_4, filtered and taken to dryness under vacuum. The aspalathin was more conveniently recovered as an amorphous white powder by evaporation to dryness under reduced pressure at 100° from solution in acetone. Yield after drying under vacuum at 100° over P_2O_5 for 2 hr.: 4·10 g., representing 20·4% (w/w) of the crude ethyl acetate-extractable material. More drastic drying resulted in a further loss in weight but discoloration of the material also took place. The compound softened at 140° and melted over the range 150–160° (decomp.), $[\alpha]_D^{20}$ 0·0° (*c* 3·20 in pyridine), +23·1±0·8° (*c* 2·42 in dimethylformamide), +34·7±0·9° (*c* 2·14 in ethanol), +58·4±0·9° (*c* 2·21 in acetone); $\lambda_{max.}$ 290 mμ in ethanol (log ε 4·20), $\lambda_{max.}$ 308 mμ in 75 mM-$AlCl_3$ in ethanol (log ε 4·32), $\lambda_{max.}$ 330 mμ in 2 mM-sodium ethoxide (log ε 4·20); infrared spectrum 3390 (OH); 2920 (aromatic C–H); 1625 (conjugated C–O, aromatic C–C); 1610, 1520, 1450 (aromatic C–C); 1360, 1280, 1160, 1110, 1080, 1035 and 820 cm.$^{-1}$ (unknown) (Found: C, 53·5; H, 5·5. $C_{21}H_{24}O_{11},H_2O$ requires C, 53·6; H, 5·5%).

No sugar could be detected when aspalathin (10 mg.) in ethanol (1 ml.) was refluxed with aq. 2N-HCl (5 ml.) for periods up to 24 hr. Fusion with dry KOH by the micromethod of Roux (1958) resulted in degradation to phloroglucinol and protocatechuic acid.

Aspalathin is readily soluble in water and other polar solvents but is insoluble in non-polar media. All attempts to crystallize the compound were unsuccessful. It was, however, pure by chromatography and had R_F values 0·37 (water), 0·63 [aq. 15% (v/v) acetic acid], 0·77 [aq. 60% (v/v) acetic acid], 0·61 [butan-1-ol–acetic acid–water (20:5:11, by vol.)] and 0·46 [phenol–water (3:1, v/v)]. Aspalathin is located on paper chromatograms as a light-brown spot under u.v. light (3650 Å). It reacts readily with

ammoniacal $AgNO_3$ (Partridge, 1948) and forms a chelate on treatment with ethanolic 1% (w/v) $AlCl_3$ (Gage, Douglass & Wender, 1951) which fluoresces an intense yellow-green under u.v. light. Other sensitive chromogenic spray reagents for this compound are bis-diazotized benzidine (Koch & Krieg, 1938) (orange-red), the $FeCl_3$–$K_3Fe(CN)_6$ reagent of Barton, Evans & Gardner (1952) (blue) and the vanillin–toluene-*p*-sulphonic acid reagent of Cartwright & Roberts (1954) (pink). Aspalathin gives a negative magnesium–HCl reduction test (Shinoda, 1928) and, although 0·5 μg. quantities of flavanones can be detected on paper chromatograms with the $NaBH_4$–$AlCl_3$ reagent (Koeppen, 1965*a*), aspalathin gives no coloration even when present in 100 μg. amounts.

Preparation of derivatives

Aspalathin nona-acetate. Aspalathin (100·3 mg.) was dissolved in a mixture of pyridine (0·4 ml.) and acetic anhydride (0·4 ml.). After 8 hr. at about 20° the solution was poured into cold water (25 ml.) and the solid which precipitated was allowed to harden. The product (169·5 mg.) crystallized from ethanol as feathery clusters of fine white needles, m.p. 153–154°; $[\alpha]_D^{25} - 35·8 \pm 0·4°$ (*c* 2·16 in acetone); $\lambda_{max.}$ 221 and 324 mμ (inflexion) in ethanol (logϵ 4·34 and 3·23 respectively); infrared spectrum 2930 (aromatic C–H); 1775, 1758 (acetyl C=O); 1710 (conjugated C–O); 1618, 1580 (inflexion), 1509 (aromatic C–C); 1430 (α-CH_2); 1372 (acetyl CH_3–); 1220, 1185 (acetate C–O–); 1112, 1045 and 908 cm.$^{-1}$ (unknown) (Found: mol.wt. 790 and C, 55·9; H, 5·0; CO·CH_3, 45·6. $C_{39}H_{42}O_{20}$ requires mol.wt. 830 and C, 56·4; H, 5·0; CO·CH_3, 46·6%).

α-Bromoaspalathin nona-acetate. The method employed was essentially that of Lorette, Gage & Wender (1951). Anhydrous conditions and the use of freshly recrystallized benzoyl peroxide (Vogel, 1957) were essential. Aspalathin acetate (250 mg.), *N*-bromosuccinimide (110 mg.) and benzoyl peroxide (6 mg.) were dissolved in a mixture of $CHCl_3$ (2 ml.) and CCl_4 (3 ml.). The solution was refluxed vigorously for 4 hr. and taken to near dryness under reduced pressure at about 18°. Ethanol (15 ml.) was added, the mixture was left for several hours at 0° and the solid was filtered off and recrystallized to constant m.p. from ethanol. The product crystallized as clusters of fine white needles. Yield after two recrystallizations and drying under vacuum to constant weight over P_2O_5 at 110°: 75 mg., m.p. 185° (Found: mol.wt. 860 and C, 51·0; H, 4·6; Br, 9·6. $C_{39}H_{41}BrO_{20}$ requires mol.wt. 909 and C, 51·5; H, 4·5; Br, 8·8%).

3,4,4′,6′-Tetra-O-methylaspalathin. Aspalathin (226 mg.) was refluxed with dimethyl sulphate (252 mg.) and anhydrous K_2CO_3 (0·7 g.) in acetone (10 ml.) for 6 hr. The solution was filtered and the residue was washed with three small portions of acetone. The filtrate and washings were combined and evaporated to dryness under reduced pressure at 70°. The methylated product was obtained as an amorphous cream-coloured powder which melted over the range 85–95°. Attempts to crystallize the compound were unsuccessful even after chromatography on 1 mm. layers of silica gel. Yield after vacuum drying over P_2O_5 to constant weight: 170 mg. (Found: C, 59·4; H, 6·2; OCH_3, 23·2. $C_{25}H_{32}O_{11}$ requires C, 59·0; H, 6·3; OCH_3, 24·4%).

2′,3,4,4′,6′-Penta-O-methylaspalathin. A dry ethereal solution of diazomethane (about 100 ml.) generated from nitrosomethylurea (6·0 g) was added to a solution of aspalathin (310 mg.) in methanol (30 ml.) at −15°. The mixture was kept at −15° for 72 hr. and then at room temperature for a further 12 hr. The solution was taken to dryness under vacuum and the residue was dissolved in $CHCl_3$ (15 ml.) and filtered. On removal of the $CHCl_3$ under reduced pressure at 50° the solute puffed up as an amorphous cream-coloured solid. Yield: 356 mg. As with the tetramethyl ether, all attempts to crystallize penta-*O*-methylaspalathin were unsuccessful. The compound softened at 75° and melted over the range 78–82°; $[\alpha]_D^{20} - 6·7 \pm 0·2°$ (*c* 3·86 in $CHCl_3$); $\lambda_{max.}$ 225 and 280 mμ in ethanol (logϵ 4·33 and 3·79 respectively); infrared spectrum 3420 (OH); 2930 (aromatic C–H); 1700 (conjugated C–O); 1620, 1600, 1515, 1462 (aromatic C–C); 1420 (α-CH_2); 1260 (aryl ether C–O–); 1236, 1205, 1140 (unknown); 1100 (aryl ether C–O–); 1028 and 810 cm.$^{-1}$ (unknown) (Found: C, 58·9; H, 6·5; OCH_3, 27·4. $C_{26}H_{34}O_{11}$ requires C, 59·8; H, 6·5; OCH_3, 29·7%).

Acetylation of the compound (120 mg.) with acetic anhydride (0·5 ml.) and pyridine (0·5 ml.) at 35° for 12 hr. yielded an amorphous white tetra-acetate which softened at 60° and melted over the range 65–68° (Found: C, 59·9; H, 6·8; OCH_3, 22·1; CO·CH_3, 25·5. $C_{34}H_{42}O_{15}$ requires C, 59·1; H, 6·1; OCH_3, 22·5; CO·CH_3 24·9%).

Oxidations of aspalathin and derivatives

Periodic acid oxidation. 2′,3,4,4′,6′-Penta-*O*-methylaspalathin was dried to constant weight over P_2O_5 under reduced pressure at 60° and oxidized by the Fleury–Lange procedure as described for orientin and iso-orientin tetramethyl ethers (Koeppen & Roux, 1965*b*). The rate of oxidation was followed by analysing samples of the reaction mixture and the blank after 7 min., 22 min., 66 min., 3 hr., 5 hr. and 24 hr. The periodate uptake/mol. of aspalathin pentamethyl ether was found to be 0·4, 0·8, 1·3, 1·7, 1·9 and 1·9 mol. respectively, thus closely paralleling the previously reported rates for tetra-*O*-methyliso-orientin (Koeppen, 1962).

The acid produced was determined by withdrawing a sample of the reaction mixture (2 ml.) after 24 hr., adding ethylene glycol (0·5 ml.), heating to 50°, allowing to cool for 10 min. and titrating with standard 0·01 N-NaOH and phenolphthalein indicator. The result, expressed as formic acid, indicated the formation of 1·0 mol./mol. of penta-*O*-methylaspalathin. The specific determination of formic acid by the procedure described (Koeppen & Roux, 1965*b*) resulted in the formation of calomel equivalent to 0·8 mol. of formic acid/mol. of penta-*O*-methylaspalathin.

Glycerol was detected as a product of HIO_4 oxidation, $NaBH_4$ reduction and acid hydrolysis of 2′,3,4,4′,6′-penta-*O*-methylaspalathin by the method described by Koeppen (1965*b*).

Ferric chloride oxidation. Oxidation of aspalathin and its pentamethyl ether by the method of Hay & Haynes (1956) as modified by Koeppen & Roux (1965*b*) resulted in the liberation of glucose. In contrast with the findings for orientin and iso-orientin (Koeppen & Roux, 1965*b*), and particularly for barbaloin (Hay & Haynes, 1956), only a trace of arabinose could be detected.

Permanganate oxidation. Under the conditions employed for orientin and iso-orientin trimethyl ethers (Koeppen & Roux, 1965*b*) both tetra- and penta-*O*-methylaspalathin

yielded veratric acid, identical with authentic veratric acid by m.p. and mixed m.p. (182°) and by infrared spectroscopy over the range 2·5–25·0 μ.

Photochemical oxidation. The following solutions were prepared in 50 ml. flasks fitted with ground-glass stoppers. (*A*) Aspalathin (75 mg.) in ethanol (25 ml.). (*B*) The same as (*A*) but the flask and stopper were painted black to exclude all light from the contents. (*C*) Aspalathin (180 mg.) in ethanol (120 ml.) was concentrated by boiling to 60 ml. The flask was filled to capacity and hermetically sealed. Solutions (*A*)–(*C*) were placed at a window which received about 5 hr. of direct sunlight/day. After 3 days solution (*A*) was slightly discoloured and after 20 days it had changed to a golden-yellow colour, whereas (*B*) and (*C*) remained colourless. Examination of samples (0·01 ml., representing 30 μg. of aspalathin) by paper chromatography in aq. 5% (v/v) acetic acid and spraying with ethanolic 1% (w/v) $AlCl_3$ soln. (Gage *et al.* 1951) revealed that (*B*) and (*C*) contained only aspalathin (R_F 0·45) whereas (*A*) contained small amounts of two additional compounds, *a* and *b*, with R_F 0·49 and 0·55 respectively. Treatment of duplicate chromatograms with $NaBH_4$ and $AlCl_3$ as described by Koeppen (1965*a*) revealed these two additional compounds as purple spots. This reaction is considered to be specific for flavanones or for dihydroflavonols which do not possess a 5-hydroxyl group or where H-bonding at this group is eliminated (Koeppen, 1965*a*).

In another experiment solution (*A*) was examined by chromatography in aq. 5% (v/v) acetic acid after 3, 6, 10, 15 and 20 days. It was thus revealed that *b* was the first-formed product whereas *a* could be detected only after 10 days. After 2 months the solution was orange–brown and no residual aspalathin was present, the main detectable products being *a* and *b* in the approximate ratio 1:2. The presence of hydroquinone markedly retarded the conversion of aspalathin into *b* or *a* whereas the hermetically sealed solution (*C*) remained colourless and completely unchanged even after 6 months. It thus appeared that the conversion of aspalathin into *b* and *a* was a photochemical oxidation. The products *a* and *b* were identical by chromatography with 2,3-dihydro-orientin and 2,3-dihydroiso-orientin respectively. The reference compounds were prepared by hydrogenation of orientin and iso-orientin, isolated from *A. linearis* (Koeppen *et al.* 1962), by modification of the procedure of Geissman & Clinton (1946) for the hydrogenation of luteolin to eriodictyol.

2,3-*Dihydro-orientin.* Orientin (10 mg.) was refluxed in ethanol (6 ml.) and water was added dropwise until the solid had dissolved completely. The solution was cooled to room temperature (18°), Adams' PtO_2 (10 mg.) was added and the mixture was hydrogenated under 1 atm. for 40 min. with constant stirring. The mixture was applied as a band along one of the short sides of a Whatman no. 3MM paper sheet (46 cm. × 57 cm.) and the chromatogram was developed by downward migration in butan-1-ol–acetic acid–water (20:5:11, by vol.). The 2,3-dihydro-orientin was located under u.v. light as a dark band of higher mobility than the main band (unchanged orientin) and its identity could be confirmed by cutting a narrow strip from the chromatogram and locating the region giving a positive $NaBH_4$–$AlCl_3$ reaction (Koeppen, 1965*a*). The band containing the 2,3-dihydro-orientin was eluted from the chromatogram with aq. 70% (v/v) ethanol and the eluate was concentrated under reduced pressure to a small volume (1–2 ml.).

2,3-*Dihydroiso-orientin.* Iso-orientin (10 mg.) dissolved in acetic acid (6 ml.) was hydrogenated with the aid of Adams' PtO_2 (10 mg.) and constant stirring under 1 atm. at 18° for 30 min. The 2,3-dihydroiso-orientin which formed was purified by chromatography as described for 2,3-dihydro-orientin.

Nuclear-magnetic-resonance spectra of aspalathin, phloretin and various derivatives

The n.m.r. spectra were recorded on Varian A–60 spectrometers by D. G. R. during the tenure of a Charles Bullard Fellowship, 1962–1963, at Harvard University and by Dr K. G. R. Pachler, Chemical Physics Group, C.S.I.R., Pretoria. Acetates were examined in deuterochloroform solution; in other cases dimethyl sulphoxide and pyridine were employed as solvents. The sample concentration was 30–100 mg. to 0·5 ml. of solution. Tetramethylsilane was used as the internal standard (τ 10·00 p.p.m.) and band positions are expressed as p.p.m. on the τ-scale. The results are summarized and compared with data for phloretin and its corresponding derivatives in Tables 1–3.

DISCUSSION

The degradation of aspalathin to phloroglucinol and protocatechuic acid on fusion with potassium hydroxide suggested that the compound was biogenetically related to the other flavonoids isolated from rooibos tea, all of which gave the same products when similarly treated (Koeppen *et al.* 1962). Analysis of the crystalline aspalathin acetate indicated a total of nine hydroxyl groups in the parent compound. This was supported by analysis of the methyl ether prepared under conditions restricted to the methylation of phenolic hydroxyl groups (cf. Hergert, Coad & Logan, 1956) and by analysis of the acetylated methyl ether, which, although amorphous, indicated that five of the hydroxyl groups in aspalathin were phenolic. The existence of the residual four as alcoholic hydroxyl groups was confirmed by the identification of a *C*-glucopyranosyl residue in aspalathin (cf. Koeppen & Roux, 1965*b*). It thus appeared that all the hydroxyl groups of the phloroglucinol and catechol nuclei were free in aspalathin and this suggested that the compound possessed a chalcone-type structure. Absorption bands at 1700 cm.$^{-1}$ and 1710 cm.$^{-1}$ in the infrared spectra of the penta-methyl ether and nona-acetate respectively, indicated the existence of a carbonyl group in aspalathin, and the ability of the compound to form a chelate with aluminium chloride which fluoresces a bright yellow–green on paper chromatograms under ultraviolet light indicated the direct attachment of the carbonyl group to the phloroglucinol nucleus (Koeppen, 1965*a*). The colourless nature of aspalathin and its single absorption maximum at 290 mμ in the ultraviolet region preclude the carbonyl group from also being conjugated with the catechol nucleus

Table 1. *τ values for various aspalathin acetates and for phloretin acetate in deuterochloroform*

Proton	τ (p.p.m.) Aspalathin nona-acetate	Phloretin penta-acetate‡	α-Bromoaspalathin nona-acetate	Penta-*O*-methyl-aspalathin tetra-acetate
3′-H	—	3·08	—	—
5′-H	3·00		2·93	3·64
2-H	2·92	2·67§	2·69	3·10
6-H				
5-H		2·93§	2·75	
3-H	—		—	—
1″-H	5·28†	—	5·26†	4·66†
α-Methylene	6·98	4·43‖	4·48‖	6·94
β-Methylene		6·55§	6·30§	
3-Acetoxyl*	7·90	—	7·81	6·24
4-Acetoxyl*	7·82	7·91	7·79	6·15
2′-Acetoxyl*	7·62	7·86	7·62	6·06
4′-Acetoxyl*	7·73	7·78	7·73	6·08
6′-Acetoxyl*		7·86		
2″-Acetoxyl	8·22	—	8·22	8·22
3″-Acetoxyl	7·94	—	7·96	7·91
4″-Acetoxyl	7·97	—	7·98	7·94
6″-Acetoxyl	8·00	—	8·00	7·97

* Methoxyl in this position in penta-*O*-methylaspalathin tetra-acetate.
† Doublet (*J* 9–10 cyc./sec.).
‡ Enol acetyl protons at τ 7·74 p.p.m.
§ Doublet (*J* 7–8 cyc./sec.).
‖ One-proton triplet (intensity, 1:2:1).

Table 2. *τ values for aspalathin, phloretin and their methyl ethers in dimethyl sulphoxide*

Proton	τ (p.p.m.) Aspalathin	Phloretin‖	3,4,4′,6′-Tetra-*O*-methylaspalathin	4,4′,6′-Tri-*O*-methylphloretin
3′-H	—	4·10	—	3·93
5′-H	4·03		3·82	
2-H	3·39*	2·96‡	3·22*	2·86‡
6-H	3·42†		3·30†	
3-H	—	3·29‡	—	3·20‡
5-H	3·53‡		3·45‡	
1″-H	5·41§	—	5·42§	—
2′-OH	−3·70	−2·30	−4·00	−3·77

* Poorly resolved doublet ($J_{meta} \approx 2$ cyc./sec.).
† Poorly resolved quartet ($J_{ortho} \approx 8$ cyc./sec., $J_{meta} \approx 2$ cyc./sec.).
‡ Doublet (J_{ortho} 8 cyc./sec.).
§ Doublet (*J* 10 cyc./sec.).
‖ cf. Batterham & Highet (1964).

and a dihydrochalcone structure for aspalathin therefore appeared to be the most feasible. This was supported by the photochemical conversion of aspalathin into 2,3-dihydroiso-orientin, which is shown to be an oxidation process.

The photochemical oxidation of aspalathin is of particular interest as similar photochemical changes involving the oxidation level of the C_3-aliphatic portion of other flavonoid compounds do not appear to have been observed. Indeed, under the conditions employed for aspalathin no conversion of phloretin (2′,4,4′,6′ - tetrahydroxydihydrochalcone) into naringenin took place. However, although no residual aspalathin could be detected after prolonged irradiation, relatively little flavanone was present, most of the original compound having

Table 3. *τ values for aspalathin and phloretin in pyridine*

Proton	τ (p.p.m.) Aspalathin	Phloretin
3′-H	—	3·57
5′-H	3·58	
1″-H	4·30*	—
α-Methylene	6·43†	6·33†
β-Methylene	6·90†	6·80†

* Doublet (*J* 10 cyc./sec.).
† Multiplet.

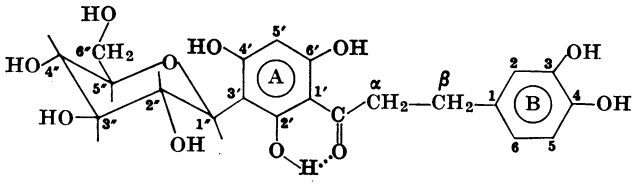
undergone further oxidative changes. It might well be that some conversion of phloretin into naringenin would be detected under more efficient photochemical conditions and experiments in this direction are, at present, in progress.

N.m.r. spectroscopy confirms the proposed *C*-glycosyldihydrochalcone structure for aspalathin. Thus, in conformity with the analyses, nine acetyl groups are indicated in the spectrum of aspalathin acetate (Table 1), the signals over the range τ 7·50–8·25 p.p.m. integrating for 27 protons. Similarly, 15 methoxyl and 12 acetyl protons are accounted for in the spectrum of penta-*O*-methylaspalathin tetra-acetate. Both spectra exhibit a four-proton signal in the region τ 7·0 p.p.m., and proof that this is due to the ethylenic grouping of aspalathin is forthcoming from the spectrum of α-bromoaspalathin nona-acetate. Thus no signals are observed in the region τ 7·0 p.p.m. in this latter compound, the residual three protons of the ethylenic group appearing as an A_2B system clearly superimposed on the proton spectrum of the glucopyranosyl residue further downfield (Table 1). The ethylenic protons of aspalathin and those of phloretin exhibit strikingly similar resonance patterns in pyridine (Table 3), indicating their basically similar environment in each compound. However, a significant difference between aspalathin and phloretin (apart from that of susceptibility to photochemical oxidation) is that acetylation of the latter under the conditions employed for the preparation of aspalathin nona-acetate resulted in the formation of an enol acetate. Thus the spectrum of phloretin acetate reveals the existence of five acetyl groups, the signals over the range τ 7·50–8·00 p.p.m. integrating for a total of 15 protons while the residual three protons on the α- and β-carbon atoms appear as an A_2B system (Table 1). The 3-hydroxyphloretin isolated by Williams (1961) yielded a penta-acetate on treatment with acetic anhydride and pyridine and this would suggest that the product, like aspalathin acetate, is not an enolate. Under the same conditions, however, phloretin is reported to form a tetra-acetate, m.p. 94–95°, whereas under the conditions of the present study a penta-acetate, m.p. 165°, was obtained.

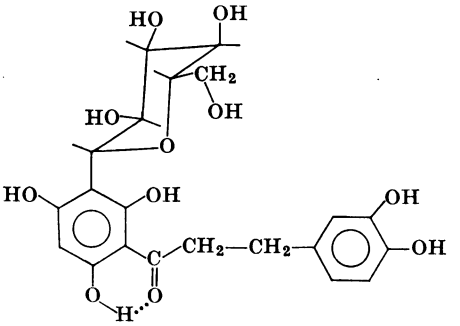
The *C*-1″ proton of aspalathin appears as a doublet (*J* 10 cyc./sec.) in the spectra of this compound and its derivatives (Tables 1–3), indicating that the compound, like other *C*-glycosylflavonoids (Horowitz & Gentili, 1964; Hillis & Horn, 1965; Koeppen & Roux, 1965*b*), possesses a β-*C*-glycosyl residue. In addition, the diamagnetic shielding of the *C*-2″ acetoxyl protons resulting in their resonance significantly further upfield from other acetyl signals (Table 1) requires the 2″-acetoxyl group to have an equatorial orientation (Hillis & Horn, 1965), which is in conformity with the existence of a *C*-β-D-glucopyranosyl residue in aspalathin.

Hay & Williams (1964) have examined various H-bonded carbonyl compounds and have found that the decrease in carbonyl stretching frequency with increase in H-bond strength is accompanied by a proportional downfield shift in the resonance position of the bonded hydroxyl proton. This general tendency is also observed for aspalathin and phloretin. Thus phloretin exhibits carbonyl absorption at 1638 cm.$^{-1}$ and 2′-hydroxyl proton resonance at τ − 2·30 p.p.m. An increase in H-bond strength on partial methylation is indicated by a decrease in both carbonyl-absorption frequency and the resonance position of the 2′-hydroxyl proton, the former occurring at 1622 cm.$^{-1}$ and the latter at τ − 3·77 p.p.m. in 4,4′,6′-tri-*O*-methylphloretin. In the infrared spectrum of aspalathin, the carbonyl absorption coincides with benzenoid absorption at 1625 cm.$^{-1}$. The 2′-hydroxyl proton resonance might therefore be expected to occur in the same region as in 4,4′,6′-tri-*O*-methylphloretin and this is indeed found to be the case (Table 2). Methylation of the remaining phenolic hydroxyl groups in aspalathin results in a further small decrease in both carbonyl-absorption frequency and position of 2′-hydroxyl proton resonance. The n.m.r. and i.r. evidence would therefore suggest that the 2′-hydroxyl proton is more strongly H-bonded in aspalathin than in phloretin. It might therefore be expected that the 2-hydroxyl group would be more resistant to methylation in the former than in the latter compound. However, the opposite has been found to be the case. Thus no difficulty was experienced in methylating all the phenolic hydroxyl groups of aspalathin by treatment with diazomethane whereas phloracetophenone and phloretin only formed di- and tri-*O*-methyl derivatives respectively under similar conditions. Similar observations have been made by Williams (1961) concerning differences in the susceptibility to methylation of the phenolic hydroxyl groups in dihydrochalcone glycosides and aglycones.

The conclusion that the 2′-hydroxyl proton is



(I)



(II)

more strongly H-bonded in aspalathin than in phloretin is, however, compatible with the known de-shielding of the 5-hydroxyl proton which occurs when a *C*-glycosyl residue is introduced in the 6- or 8-position of a 5-hydroxyflavone or flavanone (Batterham & Highet, 1964). As this effect is more pronounced when the *C*-glycosyl residue is in the *ortho*- rather than in the *para*-position (Hillis & Horn, 1965; Koeppen & Roux, 1965*b*), the H-bond might be expected to be slightly stronger in (I) than in (II). The preferred structure (I) would also account more satisfactorily for the almost exclusive formation of 2,3-dihydroiso-orientin during the initial stages of photochemical oxidation.

Ringshaw & Smith (1965) have suggested that the high τ values (3·91–4·08p.p.m.) observed for the aromatic protons of various phloracetophenones and related compounds which they examined might be of diagnostic value in recognizing the phloracetophenone moiety in natural products. While the A-ring protons of aspalathin and phloretin have τ values within or extremely close to the range quoted when the spectra are examined in dimethyl sulphoxide (Table 2), the τ values in pyridine are significantly lower (Table 3). Consideration of the results of Batterham & Highet (1964) further reveals that the resonance positions of aromatic A-ring protons do not provide a reliable indication of the existence of a phloracetophenone moiety in flavonoid compounds.

Only a few naturally occurring dihydrochalcones are known and they appear to be very much less widely distributed in plants than are other classes of flavonoid compounds (Williams, 1964). Aspalathin, identified as 3′-*C*-β-D-glucopyranosyl-2′,3,4,4′,6′-pentahydroxydihydrochalcone (I), is of particular interest in being the first example of a *C*-glycosylflavonoid of this type.

The authors are grateful to Mr P. de K. du Preez for technical assistance.

REFERENCES

Barton, G. M., Evans, R. S. & Gardner, J. A. F. (1952). *Nature, Lond.*, **170**, 249.

Batterham, T. J. & Highet, R. J. (1964). *Aust. J. Chem.* **17**, 428.

Cartwright, R. A. & Roberts, E. A. H. (1954). *J. Sci. Fd Agric.* **5**, 593.

Gage, T. B., Douglass, C. D. & Wender, S. H. (1951). *Analyt. Chem.* **23**, 1582.

Geissman, T. A. & Clinton, R. O. (1946). *J. Amer. chem. Soc.* **68**, 697.

Hay, J. E. & Haynes, L. J. (1956). *J. chem. Soc.* p. 3141.

Hay, R. W. & Williams, P. P. (1964). *J. chem. Soc.* p. 2270.

Hergert, H. L., Coad, P. & Logan, A. V. (1956). *J. org. Chem.* **11**, 304.

Hillis, W. E. & Horn, D. H. S. (1965). *Aust. J. Chem.* **18**, 531.

Horowitz, R. M. & Gentili, B. (1964). *Chem. & Ind.* p. 498.

Koch, J. E. & Krieg, W. (1938). *Chemikerztg*, **62**, 140.

Koeppen, B. H. (1962). *Chem. & Ind.* p. 2145.

Koeppen, B. H. (1965*a*). *J. Chromat.* **18**, 604.

Koeppen, B. H. (1965*b*). *Phytochemistry*, **4**, 639.

Koeppen, B. H. & Roux, D. G. (1965*a*). *Tetrahedron Lett.* no. 39, p. 3497.

Koeppen, B. H. & Roux, D. G. (1965*b*). *Biochem. J.* **97**, 444.

Koeppen, B. H., Smit, C. J. B. & Roux, D. G. (1962). *Biochem. J.* **83**, 507.

Lorette, N. B., Gage, T. B. & Wender, S. H. (1951). *J. org. Chem.* **16**, 930.

Partridge, S. M. (1948). *Biochem. J.* **42**, 238.

Ringshaw, D. J. & Smith, H. J. (1965). *Chem. & Ind.* p. 1383.

Roux, D. G. (1958). *J. Amer. Leath. Chem. Ass.* **53**, 384.

Roux, D. G. & Maihs, A. E. (1960). *Biochem. J.* **74**, 44.

Shinoda, J. (1928). *J. pharm. Soc. Japan*, **48**, 214.

Vogel, A. I. (1957). *Practical Organic Chemistry*, p. 807. London: Longmans, Green and Co. Ltd.

Williams, A. H. (1961). *J. chem. Soc.* p. 4133.

Williams, A. H. (1964). *Nature, Lond.*, **202**, 824.